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Inheritance of flour paste viscosity is associated with a rice *Waxy* gene exon 10 SNP marker☆

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ABSTRACT

Apparent amylose content is a key element for characterizing a rice (*Oryza sativa* L.) cultivar for cooking quality. However, cultivars with similar apparent amylose content can have widely varying quality attributes, including major parameters of flour paste viscosity. It has been postulated that the presence of a rice *Waxy* gene single nucleotide polymorphism (SNP) marker is associated with elevated Rapid Visco Analyser (RVA) properties in specific high amylose rice cultivars. A mapping population derived from a cross between two varieties, Cocodrie and Dixiebelle, having similar high apparent amylose contents, but with different paste viscosity properties and *Waxy* gene markers was analyzed for the genetic segregation of various pasting properties, measured with RVA instrumentation. Marker inheritance analyses revealed that the *Waxy* exon 10 SNP marker was associated with the proportion of soluble to insoluble apparent amylose and most RVA pasting measurements. *Waxy* gene markers can be used to efficiently improve the selection of rice with desirable characteristics, particularly for superior parboiling and canning quality.

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1. Introduction

Rice (*Oryza sativa* L.) flour paste viscosity measurements are a standard method to classify and grade rice end-use quality. In addition to amylose content and gelatinization temperature, paste viscosity profiles, such as those measured by Rapid Visco Analyser (RVA) techniques, are widely used to characterize rice texture, swelling, retrogradation, and canning stability (Champaign et al., 1999; Fitzgerald et al., 2003). Although apparent amylose content can explain more than half of the variation in various RVA measurements, pasting properties can vary notably from those modeled by amylose content alone (Bao et al., 2006b; Tan and Corke, 2002), particularly among high amylose content rice varieties (Gravois and Webb, 1997). It has been reported that soluble or insoluble amylose content (Bhattacharya et al., 1972; Sandhya Rani and Bhattacharya, 1995; Vandeputte et al., 2003), protein and lipid content (Fitzgerald et al., 2003; Martin and Fitzgerald, 2002; Xie et al., 2008), and amylopectin fine structure (Benmoussa et al., 2007; Ong and Blanshard, 1995; Patindol et al., 2007; although see Vandeputte et al., 2003) can have significant effects on the pasting properties of rice flour.

Early analysis on the inheritance of rice pasting properties concluded that RVA measurements are predominantly controlled by a single gene, with partial control by genes having various additive effects, and are closely linked with the inheritance of amylose content (Gravois and Webb, 1997). Although rice-growing environments can play a notable role in rice flour pasting properties (Cameron et al., 2008; Dang and Copeland, 2004), genetic effects are reported to be greater than environmental effects in heritability studies (Bao et al., 2004; Gravois and Webb, 1997).

The genetic mapping of paste viscosity characteristics has been relatively limited in scope, and mostly composed of a few studies using crosses of cultivars with widely divergent amylose content (Aoki et al., 2006; Bao et al., 2000; Larkin et al., 2003; Wang et al.,

Abbreviations: CIM, composite interval mapping; cM, centimorgans; DSC, differential scanning calorimetry; GBSS, granule bound starch synthase; LOD, logarithm of odds ratio; LS, least squares; MLE, maximum likelihood estimate; PVE, percent variance explained; QTL, quantitative trait locus; RM, rice microsatellite; RVA, Rapid Visco Analyser; SIM, simple interval mapping; SNP, single nucleotide polymorphism.

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2007). In each of these studies, the quantitative trait locus (QTL) explaining the greatest percentage of variation for paste viscosity measurements (e.g., hot paste, cool paste, setback, and breakdown viscosities) mapped to the *Waxy* locus on rice chromosome 6, which is the major locus controlling amylose content. In the only mapping study of paste viscosity made between parents having similar (intermediate) levels of amylose, no major QTL regions controlling paste viscosity characteristics were identified (Bao et al., 2002b). Even though starch structure has been seen to be significantly affected by the *Alk* gene, encoding soluble starch synthase IIa, on rice chromosome 6 (Bao et al., 2006c; Nakamura et al., 2005; Umemoto and Aoki, 2005), significant pasting property variation has not been mapped to the *Alk* gene region.

Genetic association studies using an array of rice germplasm and analyzing the correlation of paste viscosity and other end-use quality measurements with genetic marker variation, have been helpful in identifying possible genetic markers associated with paste viscosity characteristics. In a limited comparison of an array of non-glutinous (having greater than 5% apparent amylose) rice varieties, Larkin and Park (2003) surmised that single nucleotide polymorphism (SNP) variation in the *Waxy* gene at three specific positions was associated with amylose content or pasting property differences in the accessions they studied. One *Waxy* gene SNP was at the first intron splice site, well documented in previous association and inheritance studies to be directly responsible for amylose content differences observed between low amylose rice and intermediate or high amylose rice varieties (Ayres et al., 1997; Hirano et al., 1998). Larkin and Park (2003) also postulated that SNPs found at previously unreported positions within the sixth and tenth exons of the *Waxy* gene, causing amino acid substitutions, were associated (respectively) with variation in amylose content and, among a small germplasm subset studied, in paste viscosity profiles.

In a comprehensive study of 491 non-waxy (non-glutinous) rice accessions, Bao et al. (2006a) ascertained that several pasting property measurements were highly associated ($r = 0.195–0.558$) with the first intron splice site SNP of the *Waxy* gene, which also explained over 89% of the variation in amylose content. However, other polymorphisms in or near the *Waxy* gene explained additional significant RVA measurement variation (Bao et al., 2006a). Building upon the evidence that two *Waxy* gene SNPs are significantly associated with amylose content, at the first intron splice site and the sixth exon of *Waxy*, Chen et al. (2008) also associated several paste viscosity measurements with these SNPs and with amylose content. However, they also showed that variation in hot paste and cool paste viscosities were more associated with variation at the SNP in the tenth exon of the *Waxy* gene than they were with amylose content (Chen et al., 2008).

Notwithstanding, the association shown by Chen et al. (2008) between paste viscosity and the tenth exon SNP of the *Waxy* gene, it is not indisputable evidence that this trait and marker are genetically linked, because selection mechanisms, such as those applied by plant breeders, can create artificial associations between traits and markers. Without inheritance studies, it remains unknown how much linkage disequilibrium is due to genomic proximity (i.e., recombination distance) between the genetic feature controlling the measurable change of a trait and its associated marker. This study was aimed to determine the direct genetic linkage of pasting properties with the *Waxy* exon 10 SNP marker using a mapping population derived from a cross between two high-amylose rice varieties that significantly differ in their paste viscosities. Identification of genetic markers closely linked with paste viscosity measurements will benefit rice breeding programs interested in developing new cultivars using marker-aided selection (MAS) for specific processing applications or other cooking quality attributes.

2. Experimental

2.1. Materials

An F3 progeny population of 199 lines was developed by the USDA-ARS Beaumont rice breeding program from a cross between the varieties Cocodrie (CCDR, PI 606331) and Dixiebelle (DXBL, PI 595900). These two varieties have a similar apparent amylose content of 26%, but have different RVA profiles (preliminary data confirmed in this study). The parents and the progeny population were grown during the 2004 cropping season at the Texas A&M University Agricultural Research and Extension Center at Beaumont, TX. After harvest, grains were dried to 12% moisture, cleaned, and kept in air conditioned storage at room temperature until dehulling and milling. Milled samples were individually ground through a 0.40-mm screen using a cyclone sample mill (UDY, Fort Collins, CO, USA).

2.2. Degree of milling

Whole rice milling degree was determined by the method described by Hogan and Deobald (1961). Milled head rice (5 g) was refluxed with petroleum ether (Fisher, ACS grade) in a Goldfisch extraction apparatus (LabConco, MO, USA) for 30 min. Solvent was collected and evaporated at 100 °C for 30 min, and percent surface lipid content was calculated as the mass of the extracted lipid divided by the beginning total milled head rice mass × 100.

2.3. Lipid extraction from milled rice flour

Petroleum ether and 85% methanol were used, respectively, to extract free and bound lipids from rice flour of the parental lines using a Goldfisch extraction apparatus. Five replicates of the parental samples provided 3 g of rice flour that were extracted for 4 h or 16 h with 25 ml of petroleum ether or methanol, respectively. Non-solubilized portions of the extracted flours were retained, suspended in 25 ml water, and analyzed for flour paste viscosity measurements as described below.

2.4. Gelatinization temperature

Starch gelatinization temperatures of the parents were determined by differential scanning calorimetry (DSC) using a DSC6 (Perkin–Elmer Corp., Norwalk, CT) instrument. Rice flour samples of 160 mg were mixed with 320 μl of deionized water, 24 μl of the sample mixture was loaded into a stainless steel pan and weighed to the 0.1 mg level. The pan was O-ring sealed by cold welding using a Universal Sealing Press (Perkin–Elmer), equilibrated at 30 °C for 1 min, and heated from 30 to 120 °C at a rate of 5 °C/min. A sealed empty pan was used as a reference. Onset, peak and conclusion temperature and enthalpy (ΔH) of gelatinization were calculated by Pyris Thermal Analysis Manager Suite N537-0605 Version 4.0 software (Perkin–Elmer).

2.5. Apparent amylose content

Milled rice flour samples of 50 mg were weighed in duplicate, transferred to sample culture tubes and 1 ml ethanol (100%) added. The tubes were shaken gently for approximately 2–3 min. Samples were covered overnight with plastic wrap in 4 ml 1 N NaOH. The following day, 45 ml of deionized water were added to each sample. Samples were vortexed for 10–15 s and set overnight in 0.1 N NaOH at room temperature. The apparent amylose content (=total amylose) was determined using the modified iodine spectrophotometric method of Perez and Juliano (1978) with a continuous-flow analyser (Bran and Luebbe, Roselle, IL) using AACC Version 5.24